

Has Rotifera richness, abundance, and biomass been underestimated in a tropical watershed basins?

Mariane Amorim Rocha^{1,*} , Sylvia Maria Moreira Susini Ribeiro², Mauro De Melo Júnior³, Márcio Borba Da Silva⁴ and Pedro Augusto Mendes De Castro Melo⁵

¹ Laboratório Labimar, Instituto de Biologia, Universidade Federal da Bahia, Salvador, Brasil.

² Laboratório de Ecologia do Plâncton, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Bahia, Brasil.

³ Laboratório de Ecologia do Plâncton, Departamento de Biologia, Universidade Federal Rural de Pernambuco, Recife, Brasil.

⁴ Laboratório de Zoologia, Universidade Federal da Bahia, Vitória da Conquista, Brasil.

⁵ Laboratório de Fitoplâncton, Departamento de Oceanografia, Universidade Federal de Pernambuco, Recife, Brasil.

* Corresponding author: anee.bio@hotmail.com

Received: 12/11/19

Accepted: 04/06/20

ABSTRACT

Has Rotifers richness, abundance, and biomass been underestimated in a tropical watershed basins?

Suitable sampling methods must be used to accurately characterize Rotifera assemblages. The present study tested the efficiency of plankton net mesh size in Rotifera studies based on the assemblage parameters of richness, abundance, and biomass. Samples were taken in the Cachoeira River Basin (CRB), Brazil, using plankton nets with two different meshes (20 and 65 μm). A total of 69 Rotifera species were recorded, distributed among 10 families and 17 genera. We recorded 16 exclusive species in the 20 μm mesh net, representing 23.2 % of Rotifera richness. There were significant differences in the richness, abundance, and biomass captured by the two meshes. The abundances of all individual taxa were higher with the 20 μm mesh net, a pattern repeated for their frequencies of occurrence. That pattern was not observed for biomass, however, as the biomasses of eight species were greater using the 65 μm mesh net than the 20 μm mesh. The analysis of indicator species (IndVal) selected seven species as indicators of the 20 μm mesh. Smaller rotifers were underestimated in terms of all parameters analyzed (richness, abundance, and biomass) when the 65 μm mesh was used.

Key words: zooplankton, Rotifera, freshwater biodiversity, plankton net, 65 μm mesh size

RESUMO

Riqueza, abundância e biomassa de Rotifera podem ter sido subestimados em uma bacia hidrográfica tropical?

Métodos de amostragem adequados devem ser utilizados para caracterizar com precisão as assembleias de Rotifera. O presente estudo testou a eficiência do tamanho da malha de rede de plâncton nos estudos de Rotifera com base nos parâmetros de riqueza, abundância e biomassa da assembleia. As amostras foram coletadas na Bacia do Rio Cachoeira (BRC), Brasil, utilizando redes de plâncton com duas malhas diferentes (20 e 65 μm). Foram registradas 69 espécies de Rotifera, distribuídas em 10 famílias e 17 gêneros. Registramos 16 espécies exclusivas na malha de 20 μm , representando 23,2 % da riqueza de Rotifera. Houve diferenças significativas na riqueza, abundância e biomassa capturadas pelas duas malhas. A abundância de todos os táxons individuais foi maior com a malha de 20 μm , um padrão repetido por suas frequências de ocorrência. Esse padrão não foi observado para a biomassa, no entanto, como as biomassas de oito espécies foram maiores usando a malha de 65 μm do que a malha de 20 μm . A análise das espécies indicadoras (IndVal) selecionou sete espécies como indicadores da malha de 20 μm . Rotíferos menores foram subestimados em termos de todos os parâmetros analisados (riqueza, abundância e biomassa) quando a malha de 65 μm foi utilizada.

Palavras chave: zooplâncton, Rotifera, biodiversidade de água doce, rede de plâncton, malha de 65 μm

INTRODUCTION

Rotifera are cosmopolitan microorganisms that preferentially occur in continental aquatic ecosystems (Segers, 2007; Wulfken & Ahlrichs, 2012) and comprise a significant fraction of all freshwater zooplankton (Martínez *et al.*, 2000; Wen *et al.*, 2011). Rotifers play essential roles in ecosystems energy transfer, nutrient regeneration, and transport. The phylum Rotifera comprises more than 2000 described species (see Segers, 2007; Serra *et al.*, 2019), with Brazil standing out for having the largest global inventory (Aoyagui & Bonecker, 2004), 84 genera and 625 valid species (Garraffoni & Lourenço, 2012).

Although small in size, Rotifera contribute much of the freshwater aquatic zooplankton biomass and productivity (Esteves, 2011). They are often the dominant metazoans of river zooplankton and may seasonally dominate other freshwater ecosystems (Thorp *et al.*, 1994). Rotifers usually show high abundance and richness in tropical lakes (Martínez *et al.*, 2000; Wen *et al.*, 2011) and can dominate planktonic assemblages with more than 70 % of the total biomass (e.g., Hardy *et al.*, 1984).

Most studies of tropical Rotifera assemblages have focused on the spatial and temporal variability

of species compositions, richness, and diversity (Martínez *et al.*, 2000; Lucinda *et al.*, 2004; Almeida *et al.*, 2006; Serafim-Júnior *et al.*, 2010), biomass (Ulloa, 2004; Bonecker *et al.*, 2012), and secondary productivity (Casanova *et al.*, 2009). Other studies have focused on the influence of abiotic factors (Almeida *et al.*, 2006; Negreiros, 2010; Bessa *et al.*, 2011) and anthropic impacts (Bonecker *et al.*, 2009). Most of those studies have used plankton nets with mesh sizes between 63 and 80 μm .

A complete inventory of species and reliable quantitative data are needed to describe the structures and functions of zooplankton communities (Karjalainen *et al.*, 1996). Some studies have shown that mesh sizes $\geq 63 \mu\text{m}$, normally used for specific studies with crustaceans or involving all zooplankton assemblage, underestimate Rotifera abundance (Likens & Gilbert, 1970; Bottrell *et al.*, 1976; Ejsmont-Karabin, 1978). Those authors recommended a mesh size $\leq 35 \mu\text{m}$ for quantitative analyses, as microcrustaceans (Cladocera and Copepoda) show size variations superior to those found in the Rotifera group. Rotifers range in size from 50 to 2000 μm (Fontaneto, 2008), while Cladocera range from 200 to 3000 μm (Elmoor-Loureiro, 1998), and Copepoda generally from 1000 to 2000 μm (Likens, 2010). Sampling

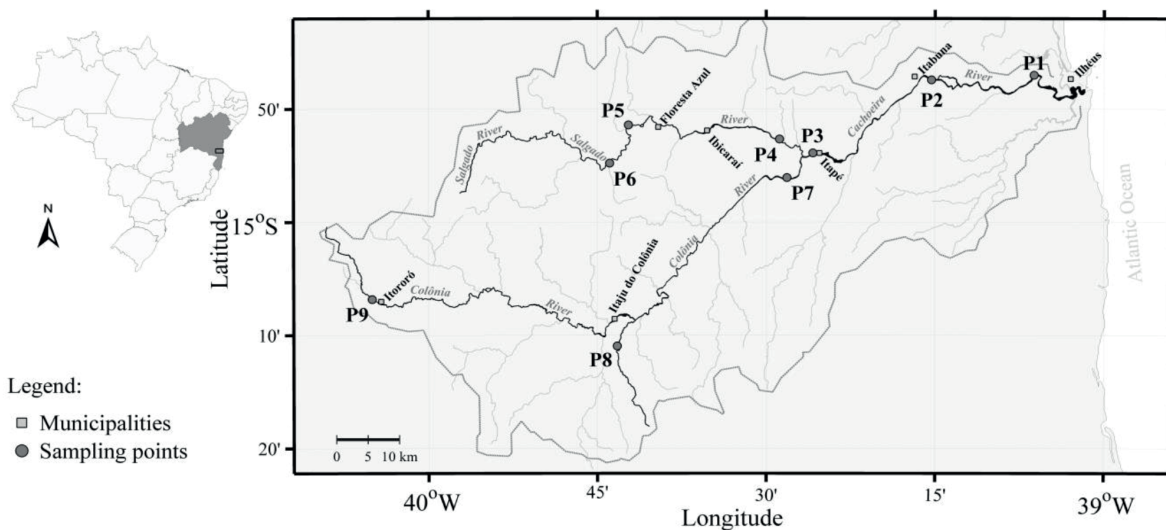


Figure 1. Location of the Cachoeira River Basin and sampling points. The main rivers (Colônia, Salgado and Cachoeira) are highlighted. *Localização da bacia do rio Cachoeira e pontos de amostragem. Os principais rios (Colônia, Salgado e Cachoeira) são destacados.*

techniques have been a major problem in studies involving Rotifera assemblages, and the use of inadequately mesh sizes can significantly influence survey results, making it difficult to conclude whether any given result is a natural feature of the population or is related to the sampling methodology used (Likens & Gilbert, 1970).

The zooplankton in tropical environments are represented by smaller species as compared to temperate regions. High temperatures can cause increases in zooplankton metabolism, sexual maturation, and reproduction, thus generating relatively smaller individuals (Kobayashi, 1997).

A study undertaken in a sub-tropical river that compared Rotifera assemblages using different size meshes, demonstrated that the roles of Rotifera in aquatic trophic webs and ecosystem processes are often underestimated (Chick *et al.*, 2010). Those authors observed that the use of a 63 μm plankton net could underestimate the abundance and biomass of Rotifera by two to three orders of magnitude – values considerably higher than suggested by previous studies (Likens & Gilbert, 1970; Bottrell *et al.*, 1976; Ejsmont-Karabin, 1978).

Although several studies have highlighted the use of smaller net meshes for ecological studies of Rotifera assemblages, none of them consider tropical environments, which are known to have smaller species. Our study therefore investigated whether the mesh sizes of plankton nets would alter the results of a Rotifera richness, abundance, and biomass surveys in a tropical basin, and possible consequences for ecological interpretations.

METHODS

Study site

The Cachoeira River Basin (CRB) is located in southern Bahia State, Brazil ($14^{\circ} 42' / 15^{\circ} 20' \text{ S}$, $39^{\circ} 01' / 40^{\circ} 091' \text{ W}$) (Fig.1). It has a drainage area of approximately 4600 km^2 (Bahia, 2001), and is the largest in the Eastern Hydrographic Basin (EHB) along the southern coast of Bahia (Lucio *et al.*, 2012).

The CRB begins at the headwaters of the Colonia River (at 800 m a.s.l.) high in the Cordillera Ouricana (Itororó municipality), and reaches

the sea near the city of Ilhéus. The Cachoeira River is formed by the confluence of the Salgado and Colônia rivers, and flows through the cities of Itapé, Itabuna, and Ilhéus. The average regional temperature there is 24.6°C , with a mean annual rainfall rate of 1500 mm in Itabuna and 2000 mm in Ilhéus (Lima *et al.*, 2010).

The water resources in the CRB are strongly influenced by pasture lands that have largely replaced the original vegetation in the Colônia and Salgado river basins, and by the growing urbanization of the cities of Itabuna and Ilhéus (De Paula *et al.*, 2012).

Sampling Strategy

Plankton samples were harvested during the day during four collection excursions: C1 (November/2014), C2 (January/2015), C3 (March/2015), and C4 (May/2015). Samples were collected at three points in each of three rivers within the CRB: the Colônia (P1, P2 and P3), Salgado (P4, P5 and P6), and Cachoeira (P7, P8 and P9) rivers (Fig.1). At each point, samples (in triplicate) were collected in the littoral zone and in the limnetic region, in order to capture the representativeness of each river section. Thus, during each campaign, 27 samples were collected (except during C3, when point 4 in the Salgado river was totally dry).

As the depths of those rivers were less than 1.5 m, sampling by towing plankton nets would not be adequate, as noted by Riccardi (2010). The samples were therefore obtained by filtering 400 L of sub-surface water at each point, with the aid of graduated pail. This volume was established in a previous campaign as the stabilization volume of the species rarefaction curve, as suggested by Omori and Ikeda (1984) for calculating adequate sample sizes. The river water was filtered through a double-mesh system with a 65 μm mesh followed by a 20 μm mesh, that was designed to allow comparisons of mesh size efficiencies and to reduce the effects of mesh clogging by suspended matter (common in those environments). After filtration, the retained material in each mesh was washed with filtered river water, and fixed in a 4 % formaldehyde solution buffered with Hexamethylenetetramine,

in 200 mL polyethylene bottles. Due to the type of sample fixation, only Monogononta rotifers were analyzed.

Laboratory Procedures and Data Treatment

For qualitative analysis of the Rotifera, the collected material was screened in Sedgwick-Rafter type chambers with the aid of an optical microscope, and the individuals found were separated in glycerin for better visualization and manipulation. When necessary, a 75 % hypochlorite solution was used for trophos extraction and the specimens stained with Bengal rose to improve visualization.

The abundances (Ind/L) of the organisms in each sample were calculated using Sedgwick-Rafter chambers from three sub-samples obtained using a Hensen-Stempell type pipette (2.5 mL) by examination under an optical microscope (Bottrell *et al.*, 1976). In cases where the subsamples contain less than 50 individuals, additional subsamples were analyzed; nonetheless, occasionally only one individual of a given

species was found. It should be stressed that the "20 μm sample" represents the sum of the Rotifera retained in both mesh types (65 and 20 μm) in the double-mesh system. That concept was used for all analyses and for all comparisons between meshes.

Biomass was estimated for all of the species in the rivers in all four sampling periods, and was calculated as the product of abundance (Ind/L) times individual dry weight (μg DW). The carbon content was considered to be 48 % of the dry weight (Andersen & Hessen, 1991), so that biomass values are expressed as $\mu\text{gC Ind}\cdot\text{L}^{-1}$.

The individual dry weights of the Rotifera species were calculated based on their biovolumes, using equations based on their geometric shapes (Table 1) (Ruttner-Kolisko, 1977). For those analyses, the lengths, widths, and heights of 30 individuals of each species per sample were measured (or the total number of individuals observed among species that did not attain that minimum abundance). The biovolume was converted to wet weight, assuming that $10^6 \mu\text{m}^3$ equals 1 μg wet weight (Bottrell *et al.*, 1976), and

Table 1. Geometric forms and mathematical equations used to determine the biovolumes of Rotifera taxa, adapted from Ruttner-Kolisko (1977), also observing the modifications suggested by Neumann-Leitão (1994). a-length; b-width; c-height. *Formas geométricas e equações matemáticas utilizadas para determinar o biovolume dos táxons de Rotifera, adaptado de Ruttner-Kolisko (1977), observando também a aplicação feita por Neumann-Leitão (1994). a-comprimento; b-largura; c-altura.*

| Taxa | Forma geométrica | Equação |
|---------------------|------------------------------|--|
| <i>Anuraeopsis</i> | Truncated trilateral pyramid | $(2*G*h)/2$, where $h = a$ and $G = b*c/2$ |
| <i>Brachionus</i> | General Ellipsoid | $(4*\pi*r^1*r^2*r^3)/3$, where $2r^1 = a$, $2r^2 = b$ and $2r^3 = c$ |
| <i>Cephalodella</i> | Cylindrical | $r^2*\pi*h$, where $r^2 = a*b/4$ and $h = c/2$ |
| <i>Cohurella</i> | Conical Cylindrical | $r^2*\pi*h + r^2*\pi*h/3$, where $h = a/2$ and $r = b/2$ |
| <i>Dipleuchnis</i> | Ellipsoid Middle | $2*\pi*r^1*r^2*r^3/3$, where $2r^1 = a$, $2r^2 = b$ and $r^3 = c$ |
| <i>Euchlanis</i> | Ellipsoid Middle | $2*\pi*r^1*r^2*r^3/3$, where $2r^1 = a$, $2r^2 = b$ and $r^3 = c$ |
| <i>Filinia</i> | Ellipsoid revolution | $4*\pi*r^1*r^2*r^3/3$, where $2r^3 = a$, $2r^1 - 2r^2 = b = c$ |
| <i>Hexarthra</i> | Cone | $r^2*\pi*h/3$, where $h = a$, $2r = b = c$ |
| <i>Keratella</i> | Parallelepiped | $r^2*\pi*h/6$, where $h = a$, $2r = b$ |
| <i>Lecane</i> | Ellipsoid Middle | $2*\pi*r^1*r^2*r^3/3$, where $2r^1 = a$, $2r^2 = b$ and $2r^3 = c$ |
| <i>Lepadella</i> | Ellipsoid Middle | $2*\pi*r^1*r^2*r^3/3$, where $2r^1 = a$, $2r^2 = b$ and $2r^3 = c$ |
| <i>Monomata</i> | Ellipsoid Middle | $2*\pi*r^1*r^2*r^3/3$, where $2r^1 = a$, $2r^2 = b$ and $r^3 = c$ |
| <i>Platyas</i> | General Ellipsoid | $4*\pi*r^1*r^2*r^3/3$, where $2r^1 = a$, $2r^2 = b$ and $2r^3 = c$ |
| <i>Polyarthra</i> | Parallelepiped | $a*b*c$ |
| <i>Squatinella</i> | Conical Cylindrical | $r^2*\pi*h + r^2*\pi*h/3$, where $h = a/2$, $r = b/2$ |
| <i>Testudinella</i> | Cylindrical | $r^2*\pi*h$, where $r^2 = a*b/4$, $c/2 = h$ |
| <i>Trichocerca</i> | Conical Cylindrical | $r^2*\pi*h + r^2*\pi*h/3$, where $h = a/2$, $r = b/2$ |

that dry weight corresponds to 10 % of the wet weight (Pace & Orcutt, 1981).

Due to the lack of formula for biovolume in the literature, the calculations for the genera *Colurella* and *Squatinella* were based on the equation proposed for *Trichocerca*; the genera *Dipleuchlanis* and *Monommata* were based on *Euchlanis*, which has a similar geometric form (Table 1).

Specific bibliographies were used to elucidate questionable species' nomenclatures, and those not identified are indicated in the list of species occurrences (Segers, 2007) (Table S1, see supplementary information, available at <http://www.limnetica.net/es/limnetica>).

Data analysis

The frequency of occurrence (FO) of a species was defined as the percentage of samples in which that specific species occurred in relation to the total sample.

The data was tested for normality using the Kolmogorov-Smirnov test. When parametric data were processed, they were compared by the t-test. When using non-parametric data, the Mann-Whitney test was used. Those tests were used to compare abundance, biomass, and richness between

the mesh sizes (20 and 65 μm). All analyses were performed using SigmaPlot 11 software.

Indicator species (IndVal) analysis (Dufrene & Legendre, 1977) was used to identify characteristic species under different conditions. This index considers both the relative abundance of a species (specificity) and its frequency of occurrence (fidelity) in a defined group. In this study, since the 20 μm samples represent the sum of the rotifers retained in the two meshes, this analysis was only used to identify species characteristic of the 20 μm mesh. The statistical significance of indicator species values was evaluated using the Monte Carlo test (1000 permutations). IndVal values were calculated using R software 2.14.1 (R Core Team, 2011), through the labdsv package (Roberts, 2015).

In all the analyses, values of $p < 0.05$ were considered significant.

RESULTS

We recorded a total of 69 Rotifera species, distributed in 10 families and 17 genera. Sixteen taxa were captured exclusively by the 20 μm mesh (Table S1, see supplementary information, available at <http://www.limnetica.net/es/limnetica>),

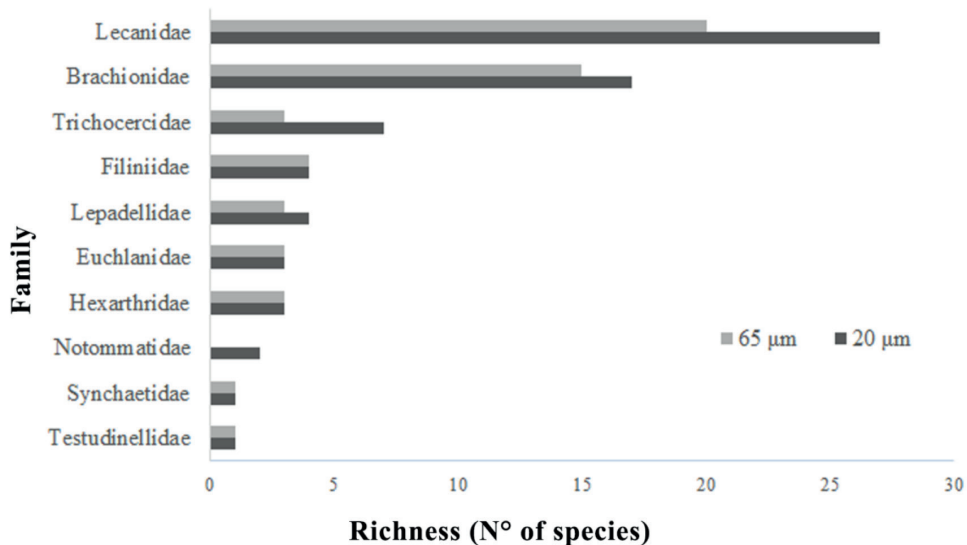


Figure 2. Numbers of Rotifera species per family in the 20 μm and 65 μm mesh nets, in the Cachoeira River Basin. *Número de espécies de Rotifera por família nas redes de 20 μm e 65 μm , na Bacia do Rio Cachoeira.*

representing 23.2 % of the Rotifera richness in this study. *Lecane bulla* (88.56 %) and *Polyarthra dolichoptera* (83.33 %) were the most frequent in the 20 μm samples, and *Lecane bulla* (80.56 %) in the 65 μm samples (Table S1).

Keratella americana, *Hexarthra intermedia braziliensis*, *Trichocerca pussila*, *Lepadella patella patella*, *Lecane lunaris lunaris*, *Colurella salina*, *Lecane quadridendata*, *Colurella obtusa obtusa*, *Polyarthra dolichoptera*, and *Lecane furcata* occurred in both mesh sizes, but showed frequencies of occurrence at least three times higher in the 20 μm mesh; *K. americana* showed a frequency of occurrence eight times higher in the 20 μm mesh. All the taxa showed higher frequencies of occurrence in the 20 μm mesh, indicating its higher efficiency.

Seven species were selected as indicators of the 20 μm mesh: *P. dolichoptera*, IndVal(%) 81.52, $p = 0.001$; *T. pussila*, IndVal(%) 63.93, $p = 0.001$; *L. patella patella*, IndVal(%) 45.77, $p = 0.001$; *C. obtusa obtusa*, IndVal(%) 33.08, $p = 0.010$; *L. lunaris lunaris*, IndVal(%) 27.66, $p = 0.030$; *K. americana*, IndVal(%) 45.50, $p = 0.001$; *Anuraeopsis fissa*, IndVal(%) 31.43, $p = 0.001$.

Rotifera richness in 20 μm mesh (69 species) was greater than in 65 μm mesh (53 species) (t-test, $t = 637$; $p < 0.001$). The most representative families were Lecanidae (27 species), Brachionidae (17 species), and Trichocercidae (7 species). Although the richness of those families was higher in the 20 μm mesh, they were represented in both meshes. Notozomatidae was exclusive to the 20 μm mesh, while Euchlanidae, Filiniidae, Hexarthridae, Lepadellidae, Synchaetidae, and Testudinellidae demonstrated the same richness in both meshes (Fig. 2).

The mean lengths of rotifers ranged from 68.3 ± 9.8 to 210.3 ± 49.6 μm throughout the study (Table S1). Species exclusive to the 20 μm mesh, belonging to the family Lecanidae and Brachionidae generally presented average lengths ≤ 100 μm (Table S1).

Abundance

The average abundance of Rotifera in the 20 μm mesh (11.95 ± 11.20 Ind/L) was 2.5 times greater

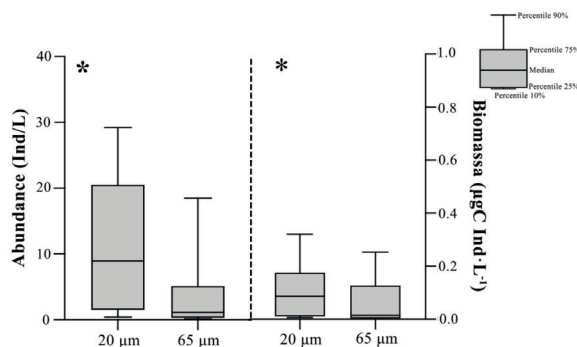


Figure 3. Abundance (Ind/L) (A) and mean biomass ($\mu\text{gC Ind}\cdot\text{L}^{-1}$) (B) of the Rotifera assemblages in the 20 μm and 65 μm mesh nets, in the Cachoeira River Basin. * indicates significant differences ($p < 0.05$). *Abundância (Ind/L) (A) e biomassa média ($\mu\text{gC Ind}\cdot\text{L}^{-1}$) (B) das assembleias de Rotifera nas redes de 20 μm e 65 μm , na Bacia do Rio Cachoeira. * indica diferenças significativas ($p < 0.05$).*

than in the 65 μm mesh (4.80 ± 8.25 Ind/L) (M-W, $U = 333.50$; $p = 0.001$) (Fig. 3). The most abundant species in the 20 μm mesh were: *P. dolichoptera*, *Brachionus urceolaris urceolaris*, *Brachionus angularis angularis*, and *Filinia terminalis*. The most abundant species in the 65 μm mesh were: *B. urceolaris urceolaris*, *F. terminalis*, *Brachionus calyciflorus*, and *Brachionus caudatus f. majusculares* (Table S1).

The abundances of all individual taxa were always greater in the 20 μm mesh, emphasizing the importance of using a small mesh for collecting all species (even the most abundant species in the 65 μm mesh).

Biomass

The mean biomass of Rotifera in the 20 μm mesh was significantly higher (0.12 ± 0.15 $\mu\text{gC Ind}\cdot\text{L}^{-1}$) than in the 65 μm mesh (0.08 ± 0.14 $\mu\text{gC Ind}\cdot\text{L}^{-1}$) (M-W, $U = 412.5$; $p = 0.0183$) (Fig. 3). *F. longiseta* showed the highest biomass value in the 20 μm mesh, followed by *Brachionus quadridentatus quadridentatus*; the reverse was observed in the 65 μm mesh. Only eight species had higher biomasses in the 65 μm mesh (Table S1), highlighting that for most species the effects of size exceed those of species densities when considering biomass.

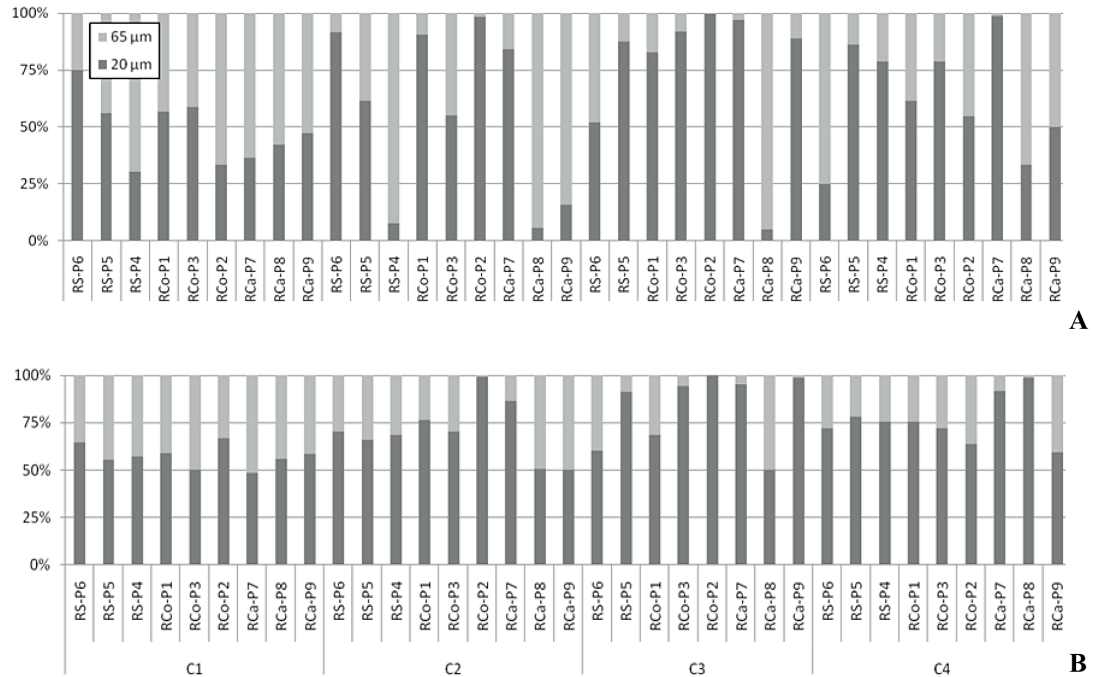


Figure 4. Contribution of the 20 µm mesh captures (dark bar) to total abundance (Ind/L) (A) and total biomass (µgC Ind·L⁻¹) (B) of the Rotifera assemblage. *Contribuição das capturas de malha de 20 µm (barra escura) para a abundância total (Ind/L) (A) e biomassa total (µgC Ind·L⁻¹) (B) da assembleia de Rotifera.*

Contribution of the 20 µm mesh to abundance and total biomass

The abundances of the Rotifera collected in the 20 µm mesh represented (on the average) 60.5 % of total abundance and 71.4 % of the total biomass sampled. Considering those results, the exclusive use of the 65 µm mesh would represent a reduction of up to 50 % in the abundance estimates in 68.6 % of the samples (24 samples); for biomass, reductions would be perceived in 97.1 % of the samples (34 samples). It should be noted that in 12 samples (34.3 % of the total) the abundance of Rotifera in the 20 µm mesh reached values higher than 80 % of the total; that same pattern was observed in 9 samples for biomass (25.7 % of the total) (Fig. 4).

DISCUSSION

The use of different mesh sizes resulted in important differences in terms of estimates of the

ecological attributes analyzed (richness, abundance, and biomass), evidencing that the 65 µm mesh underestimated Rotifera assemblages. Those results confirm data available in the literature (Chick *et al.*, 2010), which indicate that for better characterizations of Rotifera assemblage structures, plankton nets with meshes ≤ 35 µm should be used.

In the present study, a number of species would not have been recovered if only the largest mesh size (65 µm) was used, consequently underestimating Rotifera richness and contributing to inaccurate interpretations of local diversity. The species exclusive to the 20 µm mesh were represented by planktonic and non-planktonic species that had been sampled in other studies using a reduced mesh size (35 µm). *Keratella cochlearis*, *Lecane pyriformis*, *Cephalodella gibba*, *Trichocerca bicristata*, *Trichocerca elongata*, *Lecane ludwigii*, and *Lecane hamata* were observed in a tropical river (Lucinda *et al.*, 2004), while *A. fissa*, *C. gibba*, *K. cochlearis*, *L. hamata*, and *T. pussilla*

were collected in a temperate climate river (Kobayashi *et al.*, 1998). Some of those species (*A. fissa*, *K. cochlearis*, *L. hamata*, *L. ludwigii*, and *T. elongata*), however, were also sampled in studies using a larger mesh size (70 μm) in a floodplain river (Bonecker *et al.*, 2005), indicating the great variability in the sizes of individuals of different species, or the effects of clogging when filtering large volumes of water. In the present study, there were no clogging effects due to the double-mesh filtration system used.

A significant advantage of this double-mesh system is that it minimizes clogging in the smaller mesh and improves filtration efficiency. This does not occur when towing a 20 μm plankton net, however, as large cells can form a fine network inside the net and reduce its filtering efficiency and, in other cases, also trapping smaller cells that would otherwise pass through it.

The frequency of occurrence results in the two different meshes shown that the use of a 65 μm mesh was inefficient at sampling the entire Rotifera assemblage, while the 20 μm mesh permitted the sampling of smaller species. The IndVal results showed that the indicator species were those having reduced lengths (< 100 μm), such as *A. fissa*, with an average length of 76 μm (Tab. 2), evidencing the importance of using a 20 μm mesh to better evaluate ecological processes.

The families Lecanidae and Brachionidae have been observed in freshwater environments, mostly in tropical rivers (Lansac-Toha, 1997; Aoyogui & Bonecker, 2004), and significantly contribute to the richness of species sampled within different sized meshes. A 68 μm mesh net used in a study of a tropical river showed those families as having the largest numbers of species (Maia-Barbosa *et al.*, 2014), with an even greater richness of those same families being seen when using a 20 μm mesh net. The exclusive species belonging to the family Lecanidae captured in the 20 μm mesh showed mean lengths \leq 100 μm (except the species *Lecane ludwigii*, which had an average length of 118 μm) but an average width of 70 μm (which contributed to its passage through the 65 μm mesh). The species *Lecane minuta* and *Lecane monostyla* were sampled in both meshes even though they have lengths of 70 μm ; their occurrences in the 65 μm mesh was

certainly exceptional, as only one individual of each species was encountered.

The Brachionidae family was the second most representative in the present study. The species of that family exclusive to the 20 μm mesh were *A. fissa* and *K. cochlearis*, with average body lengths \leq 100 μm and average widths \leq 70 μm . Although other species show those same size variations, they were sampled in both meshes, due to wide variations in individual lengths, such as *B. angularis angularis* (min = 60 μm ; max = 260 μm), *K. americana* (min = 60 μm ; max = 160 μm), and *Keratella tropica* (min = 80 μm ; max = 130).

In general, most of the species exclusive to the 20 μm mesh had mean lengths \leq 100 μm , except for *L. ludwigii*, *Squatinella mutica mutica*, *C. gibba*, *Trichocerca tenuidens*, *T. bicristata*, and *T. elongata*, which had widths ranging from 30 to 80 μm . The narrow widths of those organisms allowed them to pass through the 65 μm mesh but become retained in the 20 μm mesh. In addition to their width values (which would contribute to their passage through the 65 μm mesh), the occurrence of *C. gibba* in the 20 μm mesh may also reflect length variations in those organisms (min = 90; max = 140).

Rotifers can show great variations in relation to their lengths and widths, and are also able to bend and contract as a result of pressure waves during filtration, so that individuals can often pass through a mesh with openings smaller than their body size – depending on their position and their reactions to touching the net (Bicudo & Bicudo, 2004).

The mean lengths of the different genera of Rotifera in the present study were close to those observed in rivers in sub-tropical regions (except for the genus *Tricocherca*, whose average lengths were almost two times as large as that seen in the present study) (Chick *et al.*, 2010). In another study undertaken in a reservoir (Ducan, 1984), representatives of the genus *Tricocherca* were reported to have average lengths similar to those found in the present study. Although the two studies were undertaken in different environments, they were both performed in the tropical region, suggesting that high temperatures may have selected for reduced sizes of the species of that genus. *Tricocherca* species are known to show wide

variations in their lengths, with *T. bicristata* in the present study being twice the size of *T. pussila*.

The Notommatidae family was exclusively encountered in the 20 μm mesh net. That family has been little-studied in tropical aquatic environments, mainly due to taxonomic difficulties (Aoyagui & Bonecker, 2004). It was represented in the present study by the species *C. gibba* and *Monommata actices*. Some authors studying Rotifera in temperate and tropical region rivers have observed that this family was represented only by *C. gibba* in 35 μm mesh samples (Kobayashi *et al.*, 1998; Lucinda *et al.*, 2004). In other studies, in different tropical aquatic environments, the species *M. actices* was collected using a net mesh of 30 μm (Meas & Sor, 2014). The Notommatidae family has been identified in some studies with mesh sizes greater than 55 μm , but represented by species different from those found in the present study (Casanova *et al.*, 2009; Lansac-Tôha *et al.*, 2009).

If we had used only a net mesh of 65 μm in the present study, only 39.47 % of the local abundance would had been revealed, a significant underestimation of the real assemblage composition (Fig. 4a). Those results corroborate studies that reported 65 μm mesh nets as underestimating Rotifera assemblages (Likens & Gilbert, 1970; Bottrell *et al.*, 1976; Ejmont-Karabin, 1978). Those authors concluded that Rotifera abundance in meshes $\geq 63 \mu\text{m}$ can vary from 28 % to 66 % of the abundance determined with meshes $\leq 35 \mu\text{m}$. Those who results confirm the observation (Pace *et al.*, 1992; Thorp & Mantovani, 2005) that river zooplankton assemblages are mainly composed of small species, and that zooplankton growing in higher temperatures environments are generally smaller than those growing at lower temperatures, due to the shorter generation times of the former (Gillooly *et al.*, 2000). Thus, there is clear need to use smaller mesh sizes in tropical regions to avoid distorted results and inaccurate ecological interpretations.

Similarly, the total estimated biomass for the CRB if only the 65 μm mesh had been used, would only represent 28 % of the true Rotifera biomass, evidencing the ecological importance of small organisms that are often neglected in tropical studies.

Implications for ecological studies in tropical rivers

The compositions of zooplankton assemblages in tropical environments are greatly affected by predators (Zaret, 1975). Another factor that appears to contribute to the dominance of small Rotifera in tropical rivers is resource availability. High productivity environments allow the development of organisms having reduced sizes (Masson *et al.*, 2004), with the size variations of zooplanktonic organisms being directly related to physical, chemical, and biotic factors.

The presence of large populations of small organisms must presumably be associated with their high efficiency in assimilating even smaller organisms, such as bacteria, nanophytoplankton, and small planktonic protozoans (Bonecker *et al.*, 2012).

However, to evaluate that hypothesis, additional studies will be needed that address parameters of Rotifera predation and the availability of appropriately sized resources in the region.

There are many sampling methods available for the quantitative analysis of zooplankton, although plankton nets are most commonly used (Sameoto *et al.*, 2000). Among the problems that influence efficient plankton sampling are the escape of zooplankton through the mesh net, and clogging. The main problem facing the use of small-mesh plankton nets are their filtering efficiencies (Tranter & Heron, 1967; Smith *et al.*, 1968) due to clogging of the mesh pores (Favoreto *et al.*, 2009). Studies of Rotifera assemblages in eutrophic lakes have shown that the apertures of small meshes rapidly become clogged and their filtration efficiencies are reduced (Likens & Gilbert, 1970). The use of a graduated bucket and a double-mesh filtration setup in the present study was well-adapted to local water depths and avoided the problem of clogging.

Likens & Gilbert (1970) pointed out that the use of small mesh size nets ($\leq 35 \mu\text{m}$) can still adequately sample various populations of zooplankton even when it is necessary to filter large volumes of water in the field. Although we did not evaluate the functionality of 35 to 37 μm meshes, species such as *A. fissa*, *C. obtusa*, *C. salina*, *K. americana*, *K. tropica*, *L. imbricata*, *P.*

dolycoptera, *T. fusiforme*, and *T. pussila* would be largely lost if a 35 µm mesh was used, the 20 µm mesh would, however, trap them efficiently as their minimum lengths/widths are proximately 40 µm. According to Milroy (2015), mesh openings must be equivalent to at least 75 % of the sizes of the individuals to be captured in order to be able to efficiently collect them, so that the use of a 35 µm mesh is only suitable for individuals larger than 50 µm.

CONCLUSION

It is clear that the use of inadequate sampling methodologies would contribute to the misinterpretation of Rotifera assemblages in aquatic ecology studies. The 65 µm mesh used in our study would not have sampled smaller species, resulting in gross underestimates of the main ecological attributes of the Rotifera assemblages (richness, abundance, and biomass).

Even though no comparison were made to towed plankton net samples, our results suggest that the 20 µm mesh collected by a double-mesh filtration setup is quite efficient and represents an available option for that kind of environment in terms of avoiding mesh clogging. Future studies comparing these two methods (double-mesh system and plankton net) should reinforce that observation.

ACKNOWLEDGEMENTS

The first author thanks CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the scholarship granted, and the logistical support of the Plankton Ecology Laboratory (UESC) and the Aquatic Biogeochemistry Laboratory (UESC). This work was supported by FAPESB (Fundação de Amparo à Pesquisa do Estado da Bahia) which financed the project (FAPESB/UESC 005/2012) and the State University of Santa Cruz (UESC 00220.1100.1360, coordinated by Daniela Mariano Lopes da Silva).

REFERENCES

ALMEIDA, V. L. S., M. E. L. LARRAZÁBAL, N.A. M. & M. MELO JUNIOR. 2006. Rotif-

era das zonas limnética e litorânea do reservatório de Tapacurá, Pernambuco, Brasil. *Iheringia, Série Zoologia*, 96: 445-451. DOI: 10.1590/S0073-47212006000400009

- ANDERSEN, T. & D. O. HESSEN. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology Oceanography*, 36: 807-814. DOI: 10.4319/lo.1991.36.4.0807
- AOYAGUI, A. S. M. & C. C. BONECKER. 2004. Rotifers in different environments of the Upper Paraná River floodplain (Brazil): richness, abundance and the relationship with the connectivity. *Hydrobiologia*, 522: 281-290. DOI: 10.1023/b:hydr.0000029980.49859.40
- BAHIA. 2001. *Secretaria de Recursos Hídricos. Programa de recuperação das bacias hidrográficas dos Rios Cachoeira e Almada. Diagnóstico Regional. Caracterização Hidrológica. Ilhéus-BA. SRH/UESC.*
- BESSA, G. F., L. C. G. VIEIRA, L. M. BINI, D. F. REIS & P. B. MORAIS. 2011. Concordance patterns in zooplankton assemblages in the UHE - Luís Eduardo Magalhães reservoir in the Mid-Tocantins river, Tocantins State, Brazil. *Acta Scientiarum Biological Sciences*, 33. DOI: 10.4025/actascibiolsci.v33i2.7336
- BICUDO, C. E. D. M. & D. D. C. BICUDO. 2004. *Amostragem em limnologia*. RiMa, São Carlos.
- BONECKER, C. C., C. L. DA COSTA, L. F. M. VELHO & F. A. LANSAC-TÔHA. 2005. Diversity and abundance of the planktonic rotifers in different environments of the Upper Paraná River floodplain (Paraná State – Mato Grosso do Sul State, Brazil). *Hydrobiologia*, 546: 405–414. DOI: 10.1007/s10750-005-4283-2
- BONECKER, C. C., A. S. M. AOYAGUI & R. M. SANTOS. 2009. The impact of impoundment on the rotifer communities in two tropical floodplain environments: interannual pulse variations. *Brazilian Journal of Biology*, 69: 529-537. DOI: 10.1590/S1519-69842009000300008
- BONECKER, C. C., F. AZEVEDO & N. R. SIMOES. 2012. Zooplankton body-size structure and biomass in tropical floodplain lakes: relationship with planktivorous fishes. *Acta*

- Limnologica Brasiliensia*, 23: 217-228. DOI: 10.1590/S2179-975X2012005000005
- BOTTRELL, H. H., A. DUNCAN, Z. M. GLIWICZ, E. GRYGIEREK, A. HERZIG, A. HILLBRICHTILKOWSKA, H. KURASAWA & P. LARSSON. 1976. A view of some problems in zooplankton production studies. *Norwegian Journal of Zoology*, 24: 419-456.
- CASANOVA, S. M. C., E. A. PANARELLI & R. HENRY. 2009. Rotifer abundance, biomass, and secondary production after the recovery of hydrologic connectivity between a river and two marginal lakes (São Paulo, Brazil). *Limnologica*, 39: 292-301. DOI: 10.1016/j.limno.2009.06.008
- CETESB. 1978. *Determinação do zooplâncton marinho: métodos qualitativos e quantitativos*, Governo do Estado de São Paulo, Companhia de Tecnologia de Saneamento Ambiental, Normatização Técnica.
- CHICK, J. H., A. P. LEVCHUK, K. A. MEDLEY & J. H. HAVEL. 2010. Underestimation of rotifer abundance a much greater problem than previously appreciated. *Limnology Oceanography: Methods*, 8: 79-87. DOI: 10.4319/lom.2010.8.0079
- DE PAULA, F. C. F., D. M. L. SILVA & C. M. SOUZA. 2012. Tipologias Hidroquímicas das Bacias Hidrográficas do Leste da Bahia. *Revista Virtual Química*, 4: 365- 373. DOI: 10.5935/1984-6835.20120028
- DUCAN, A. 1984. Assessment of factors influencing the composition, body size and turnover rate of plankton in Parakrama Samudra, an irrigation reservoir in Sri Lanka. *Hydrobiologia*, 113: 201-215.
- DUFRENE, M. & P. LEGENDRE. 1997. Species Asemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. *Ecological Monographs*, 67: 345-366. DOI: 10.2307/2963459
- EJSMONT-KARABIN, J. 1978. Studies on the usefulness of different mesh-size plankton nets for thickening zooplankton. *Ekologia polska-polish Journal of ecology*, 26: 479-490.
- ELMOOR-LOUREIRO, L. M. A. 1988. O gênero *Bosmina* (Cladocera) na região do distrito federal. *Acta Limnologica Brasiliensia*, 11: 501- 512.
- ESTEVES, F. A. 2011. *Fundamentos de Limnologia*. Interciência, Rio de Janeiro.
- FAVORETO, L. R., G. PERBICHE-NEVES, M. SERAFIM-JÚNIOR & L. P. SARTORI. 2009. Selectivity of plankton nets over planktonic Copepoda in two sub-tropical estuaries. *Acta Limnologica Brasiliensia*, 21: 67-77.
- FONTANETO, D., W. H. SMET & G. MELONE. 2008. Identification key to the genera of marine rotifers world wide. *Meiofauna Marina*, 16: 75-99.
- GARRAFFONI, A. R. S. & A. P. LOURENÇO. 2012. Synthesis of Brazilian Rotifera: An updated list of species. *Check List*, 8: 375-407. DOI: 10.15560/8.3.375
- GILLOOLY, J. F. 2000. Effect of body size and temperature on generation time in zooplankton. *Journal of Plankton Research*, 22: 241-251. DOI: 10.1093/plankt/22.2.241
- GLIWICZ, Z. 1969. Share of algae, bacteria and trypton in the food of the pelagic zooplankton of lakes with various trophic characteristics. *Bulletin Academia Polonia Sciences*, 17: 159-165
- HARDY, E. R., B. ROBERTSON & E. KOSTE. 1984. About the relationship between the zooplankton and fluctuating water levels of lago Camaleão, Central Amazonian várzea Lake. *Amazoniana*, 9: 43-52
- JUDAY, C. 1916. Limnological apparatus. *Transactions of the Wisconsin Academy of Sciences*, 18: 566-592
- KARJALAINEN, J., M. RAHKOLA, M. VILJANEN, I. N. ANDRONIKOVA & V. A. AVINSKII. 1996. Comparison of methods used in zooplankton sampling and counting in the joint Russian- Finnish evaluation of the trophic state of Lake Ladoga. *Hydrobiologia*, 322: 249-253
- KOBAYASHI, T. 1997. Associations between environmental variables and zooplankton body masses in a regulated Australian river. *Australian Journal of Marine & Freshwater Research*, 48: 523-529. DOI: 10.1071/MF96081
- KOBAYASHI, T., R. J. SHIEL, P. GIBBS & P. I. DIXON. 1998. Freshwater zooplankton in the Hawkesbury-Nepean River: comparison of assemblage structure with other rivers.

- Hydrobiologia*, 377: 133-145
- LANSAC-TÔHA, F. A., C. C. BONECKER, L. F. M. VELHO & A. F. LIMA. 1997. Composição, distribuição e abundância da comunidade zooplancônica, p. 177-155. In VAZ-ZOLER, A. E. A., A. A. Agostinho & Hahn. Planície de inundação do Alto rio Paraná: aspectos físicos, biológicos e socioeconômicos. Maringá, Eduem.
- LANSAC-TÔHA, F. A., C. C. BONECKER, L. F. M. VELHO, N. R. SIMÕES, J. D. DIAS, G. M. ALVES & E. M. TAKAHASHI. 2009. Biodiversity of zooplankton communities in the Upper Paraná River floodplain: interannual variation from long-term studies. *Brazilian Journal of Biology*, 69. DOI: 10.1590/S1519-69842009000300009
- LIKENS, G. E., J. J. GILBERT. 1970. Notes on quantitative sampling of natural populations of planktonic rotifers. *Limnology Oceanography*, 15: 816-820. DOI: 10.4319/lo.1970.15.5.0816
- LIKENS, G. E. (2010) *Plankton of Inland Waters*. Elsevier.
- LIMA, M. C., M. F. L. SOUZA, G. F. EÇA & M. A. M. SILVA. 2010. Export and retention of dissolved inorganic nutrients in the Cachoeira River, Ilhéus, Bahia, Brazil. *Journal of Limnology*, 69: 138-145. DOI: 10.4081/jlimnol.2010.138
- LUCINDA, I., H. MORENO, M. G. G. MELÃO & T. MATSUMURA-TUNDISI. 2004. Rotifers in freshwater habitats in the Upper Tietê River Basin, São Paulo State, Brazil. *Acta Limnologica Brasiliensia*, 16: 203-224.
- LUCIO, M. Z. T. P. Q., S. S. SIMONE & D. M. L. SILVA. 2012. Hydrochemistry of Cachoeira River (Bahia State, Brazil). *Acta Limnologica Brasiliensia*, 24: 181-192. DOI: 10.1590/S2179-975X2012005000037
- MAIA-BARBOSA, P. M., R. M. MENENDEZ, D. G. F. PUJONI, A. AOKI & A. R. BARBOSA. 2014. Zooplankton (Copepoda, Rotifera, Cladocera and Protozoa: Amoeba Testacea) from natural lakes of the middle Rio Doce basin, Minas Gerais, Brazil. *Biota neotropica*, 14: 1-20. DOI: 10.1590/S1676-06034040
- MARTÍNEZ, J. C. C., A. CANESIN & C. C. BONECKER. 2000. Species composition of rotifers in different habitats of an artificial lake, Mato Grosso do Sul State, Brazil. *Acta Scientiarum*, 22: 343-346.
- MASSON, S., B. PINEL-ALLOUL & P. DUTILLEUL. 2004. Spatial heterogeneity of zooplankton biomass and size structure in southern Québec lakes: variation among lakes and within lake among epi-, meta- and hypolimnion strata. *Journal of Plankton Research*, 26: 1441-1458.
- MEAS, S. & R. SOR. 2014. New Records of Rotifer Fauna in the Upper Cambodian Mekong River Basin. *International Journal of Environmental and rural development*, 5. DOI: 10.13140/rg.2.1.3034.4805
- NEGREIROS, N. F., M. J. SANTOS-WISNIEWSKI, R. M. SANTOS & O. ROCHA. 2010. The influence of environmental factors on the seasonal dynamics and composition of Rotifera in the Sapucaí River arm of Furnas Reservoir, MG, Brazil. *Acta Limnologica Brasiliensia*, 10. DOI: 10.1590/S1676-06032010000400023
- NEUMANN-LEITÃO, S. 1994. Impactos Antrópicos na comunidade zooplancônica estuarina. Porto de Suape - PE- Brasil. 1994. 273 f. Tese (Doutorado em Ciências da Engenharia Ambiental)- Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos.
- PACE, M. L. & J. D. J. R. ORCUTT. 1981. The relative importance of protozoans, rotifers and ciliate in a freshwater zooplankton assemblage. *Limnology Oceanography*, 26: 822-830. DOI: 10.4319/lo.1981.26.5.0822
- PACE, M. L., S. E. G. FINDLAY & D. LINKS. 1992. Zooplankton in advective environments: The Hudson River assemblage and a comparative analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 49: 1060-1069. DOI: 10.1139/f92-117
- RICCARD, N. 2010. Selectivity of plankton nets over mesozooplankton taxa: implications for abundance, biomass and diversity estimation. *Journal of Limnology*, 69: 287-296. DOI: 10.3274/jl10-69-2-10
- RUTTNER-KOLISKO, A. 1977. Suggestions for biomass calculation of planktonic rotifers. *Archiv für Hydrobiologie*, 8: 71-77.

- SAMEOTO, D., P. WIEBE, J. RUNGE, L. POSTEL, J. DUNN, C. MILLER & S. COOMBS. 2000. Collecting zooplankton. In Harris, R., Wiebe, P., Lenz, J., Skjoldaland, H. R. and Huntley, M. (eds), Zooplankton methodology manual. Academic Press, London.
- SEGERS, H. 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa*, 1564: 1-104.
- SERAFIM-JÚNIOR, M., G. PERBICHENEVES, L. BRITO, A. GHIDINI, A. & S. M. C. CASANOVA. 2010. Variação espaço-temporal de Rotifera em um reservatório eutrofizado no sul do Brasil. *Iheringia, Série Zoologia*, 100: 233-241. DOI: 10.1590/S0073-47212010000300008
- SERRA, M., E. M. GARCÍA-ROGER, R. ORTELLS & M. J. CARMONA. 2019. Cyclically parthenogenetic rotifers and the theories of population and evolutionary ecology. *Limnetica*, 38: 67-93. DOI: 10.23818/limn.38.13
- SMITH, P. E., R. COUNTS & R. I. CLUTTEK. 1968. Changes in filtering efficiency of plankton nets due to clogging under tow. *Journal du Conseil/Conseil Permanent International pour l'Exploration de la Mer*, 32: 232-248.
- THORP, J. H., A. R. BLACK, K. H. HAAG & J. D. WEHR. 1994. Zooplankton Assemblages in the Ohio River: Seasonal, Tributary, and Navigation Dam Effects. *Canadian Journal of Fisheries and Aquatic Sciences*, 51: 1634-1643. DOI: 10.1139/f94-164
- THORP, J. H. & S. MANTOVANI. 2005. Zooplankton of turbid and hydrologically dynamic prairie rivers. *Freshwater Biology*, 50: 1474-1491. DOI: 10.1111/j.1365-2427.2005.01422.x
- TRANter, D. J. & A. C. HERON. 1967. Experiments on filtration plankton nets. *Australian Journal of Marine and Freshwater Research*, 18: 89-111. DOI: 10.1071/MF9670089
- ULLOA, V. 2004. Density and biomass of planktonic rotifers in different habitats in upper Paraná River (PR, Brazil). *Acta Limnologica Brasiliensia*, 16: 281-292.
- WANG, S., X. PING, S. WU & W. HAIJUN. 2007. Crustacean zooplankton size structure in aquaculture lakes: is larger size structure always associated with higher grazing pressure? *Hydrobiologia*, 575: 203-209. DOI: 10.1007/s10750-006-0394-7
- WEN, X. L., Y. L. XI, F. P. QIAN, G. ZHANG & X. L. XIANG. 2011. Comparative analysis of rotifer assemblage structure in five subtropical shallow lakes in East China: role of physical and chemical conditions. *Hydrobiologia*, 661: 303-316. DOI: 10.1007/s10750-010-0539-6
- WULFKEN, D. & W. H. AHLRICHS. 2012. The ultrastructure of the mastax of *Filinia longiseta* (Flosculariaceae, Rotifera): Informational value of the trophi structure and mastax musculature. *Zoologischer Anzeiger*, 251: 270-278. DOI: 10.1016/j.jcz.2012.02.001
- ZARET, T. M. 1975. Strategies for existence of zooplankton prey in homogenous environments. *Verh. Internat. Verein Limnol.*, 19, 1484-1489.